Synthesis of Isosteric Analogues of Nicotinamide Adenine Dinucleotide Containing C-Nucleotide of Nicotinamide or Picolinamide[1]

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Two isosteric analogues of nicotinamide adenine dinucleotide, C-NAD (11) and C-PAD (12), in which the nicotinamide riboside portion is replaced by a C-nucleoside, were synthesized from 5-(β-D-ribofuranosyl)nicotinamide (7) and 6-(β-D-ribofuranosyl)picolinamide (8), respectively. Nucleoside 7 was prepared from the 2,3-*O*-isopropylidene-5-*O*-(tetrahydropyranyl)-D-ribonolactone (13) and 3-cyano-5-lithiopyridine as reported earlier. Nucleoside 8 was obtained by conversion of the bromo function of the 6-(2,3:4,5-di-*O*-isopropylidene-D-*altro*-pentitol-1-yl)-2-bromopyridine (14) into a carboxamido group followed by mesylation of the anomeric hydroxyl group to give derivative 18. Treatment of 18 with $CF_3COOH/CHCl_3$ caused deisopropylidenation with simultaneous cyclization into the desired 6-(β-D-ribofuranosyl)picolinamide (8). NAD analogues, C-NAD (11) and C-PAD (12), were synthesized by imidazole-catalyzed coupling of the corresponding 5′-monophosphates of 7 and 8 with the adenosine-5′-monophosphate. Dinucleotide 11 was found to inhibit the proliferation of L1210 cells (IC_{50} = 7 μM) and to be a good competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH, ID_{50} = 20 μM) as well as bovine glutamate dehydrogenase (GDH, K_i = 15 μM). Interestingly, C-NAD (11) caused extremely potent noncompetitive inhibition of horse liver alcohol dehydrogenase (ADH, K_i = 1.1 nM), whereas C-PAD (12) was found to be a much less potent competitive inhibitor (K_i = 20 μM) of ADH.

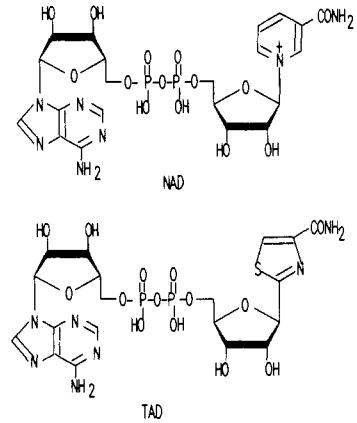
Introduction

Nicotinamide adenine dinucleotide (NAD)[2,3] is essential as a cofactor in biological hydride-transfer reactions and as ADP-ribose donor in posttranslational modification of a variety of proteins. Recently, a synthesis of numerous chemically modified NAD analogues acting as potent inhibitors of dehydrogenases, NAD-glycohydrolases, and ADP-ribosyltransferases have been reported.[4-11]

The most extensively studied thiazole-4-carboxamide adenine dinucleotide (Chart I, TAD),[12] which contains tiazofurin (TF) instead of nicotinamide riboside, is a potent inhibitor of inosine monophosphate dehydrogenase (IMPDH).[13] This enzyme catalyzes the conversion of inosine monophosphate (IMP) to xanthosine monophosphate (XMP) in the *de novo* biosynthesis of guanylic acid. The level of IMPDH activity was found to be much greater in several tumors as compared to normal tissues.[14-18] IMPDH was suggested, therefore, to be an important target in anticancer chemotherapy.[19] Recently, the reduction in the levels of guanine nucleotide as a result of IMPDH inhibition has been shown to induce differentiation in several cell lines.[20]

Tiazofurin, 2-(β-D-ribofuranosyl)thiazole-4-carboxamide (Chart II, 2), has demonstrated significant antitumor activity in a number of tumor systems and underwent clinical trials.[21-23] This C-nucleoside penetrates cell membranes and then is phosphorylated by adenosine kinase and/or by 5′-nucleotidase[24,25] to the tiazofurin 5′-monophosphate (TFMP), which is further coupled with

Chart I



adenosine 5′-monophosphate (AMP) by NAD-pyrophosphorylase to give TAD. The latter is a more potent inhibitor of IMPDH than either TFMP or TF itself.

Since the discovery of oncolytic properties of TF, other C-nucleoside analogues have been studied. Selenazofurin (3), an analogue containing selenium instead of sulfur, was synthesized,[26] and its corresponding dinucleotide anabolite (SAD) was found to be even more cytotoxic than TF.[27] In contrast, isosteric replacement of the sulfur or selenium with oxygen, as in oxazofurin (1),[28] abolished the antitumor activity. Arabino and xylo congeners of TF show no cytotoxicity.[29,30] The 5-(β-D-ribofuranosyl)-1,2,4-oxadiazole-3-carboxamide (4) caused only weak inhibition of leukemia L1210 and P388 (ca. 40% at 100 μM).[31] The synthesis of the 2-(β-D-ribofuranosyl)pyrimidine-4-carboxamide (5) and its corresponding NAD analogue has been published, but the antitumor activity of these compounds has not been reported.[32] An imidazole C-nu-

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Chart II

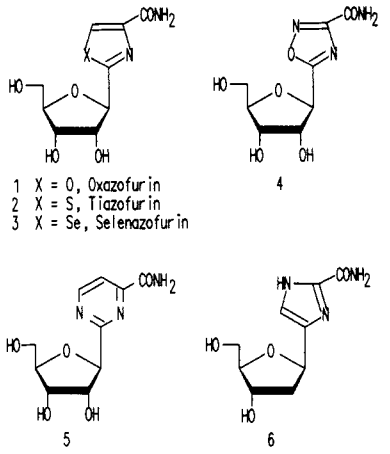
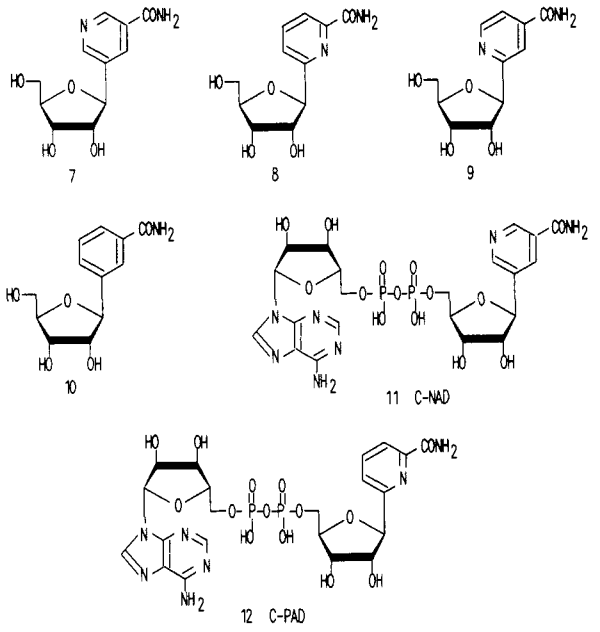


Chart III



cleoside, 4-(2-deoxy-β-D-ribofuranosyl)imidazole-2-carboxamide (**6**), has also been prepared recently.[33]

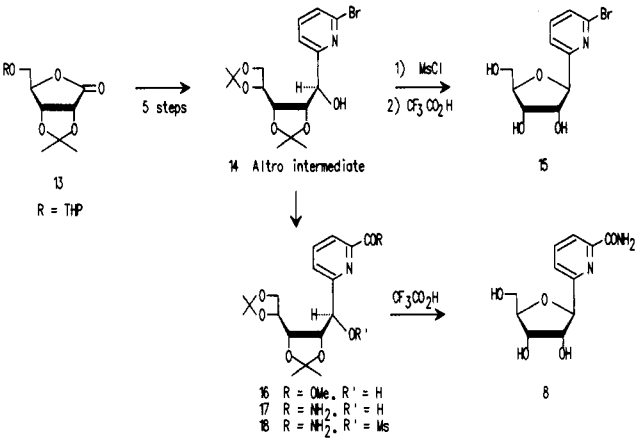
Results and Discussion

In this laboratory we initiated a search for NAD analogues which have close structural similarity to the natural coenzyme and may therefore be even more potent and specific inhibitors of IMPDH than TAD.

We have reported the synthesis of 5-β-D-ribofuranosylnicotinamide[34,35] (Chart III, **7**) and 6-β-D-ribofuranosylpicolinamide[36] (**8**) as the C-nucleoside isosteres of nicotinamide riboside. Later, 2-β-D-ribofuranosylisonicotinamide (**9**)[37] was synthesized by others. Compounds **7–9** showed weak inhibitory activity against L1210, P-815, HL-60, CCRF-CEM, MOLT/4F, and MT-4.[34,36,37] This is in sharp contrast to the very recently reported 3-β-D-ribofuranosylbenzamide[38] (**10**), which showed extremely high toxicity at nanomolar concentration to S49.1 lymphoma cells. Interestingly, benzoic acid riboside is almost nontoxic to the cells.[38]

All these studies indicate that either compounds **7–9** are not efficiently converted into the corresponding NAD analogues by cellular enzymes or NAD analogues containing **7–9** serve as weak inhibitors of IMPDH. In order to clarify this problem, we report herein the synthesis of two NAD analogues containing nucleosides **7** and **8** instead of nicotinamide riboside and the results of our preliminary studies on their inhibitory activity against IMPDH and some cellular dehydrogenases.

Scheme I



Nucleoside **7** was prepared from the 2,3-*O*-isopropylidene-5-*O*-(tetrahydropyranyl)-D-ribonolactone (**13**) and 3-cyano-5-lithiopyridine as reported earlier.[35] Although we condensed **13** with 2-bromo-6-lithiopyridine in the same manner and obtained **15**[36] (Scheme I) we did not convert it into the corresponding carboxamido derivative **8**. Since protection of the hydroxyl groups of **15** should be necessary for such conversion, we employed the protected precursor of **15**, the *altro* derivative **14**,[36] as the starting material. Thus, lithiation, carboxylation, and esterification with diazomethane afforded the corresponding methyl picolinate **16**, which was treated with NH_3/MeOH to give carboxamide derivative **17**. Conversion of **17** into the mesylate **18** followed by solvolysis with CF_3COOH afforded the desired 6-(β-D-ribofuranosyl)picolinamide (**8**) in 14.3% yield from **14**. In the same manner the α-anomer of **8** was prepared from the *allo* isomer of **14**.

C-Glycosidic nicotinamide and picolinamide adenine dinucleotide (**11**, C-NAD, and **12**, C-PAD, respectively) were synthesized by the imidazole-catalyzed coupling[10] of the corresponding 5′-monophosphates of **7** and **8** with AMP.

Preliminary studies of IMPDH (from L1210 cells) inhibitory activity showed 50% inhibition (ID_{50}) at the concentration of 20 μM C-NAD. It was also found that C-NAD inhibits the proliferation of L1210 cells by 50% (IC_{50}) at the concentration of 7 μM. Thus, C-NAD is a potent inhibitor of the enzyme, although not as good as TAD (K_i = 0.12 μM).[9] It can be therefore concluded that the weak inhibitory activity of the parent nucleoside **7** is due to its unfavorable metabolism.

Inhibition of two cellular NAD-dependent dehydrogenases, horse liver alcohol dehydrogenase (ADH) and bovine glutamate dehydrogenase (GDH), by C-NAD and C-PAD were also examined. Preliminary results for C-PAD indicated competitive inhibition of ADH with respect to NAD, with K_i = 20 μM. Interestingly, the results for C-NAD showed competitive inhibition of GDH (K_i = 15 μM), but noncompetitive inhibition of ADH, with K_i = 1.1 nM. Vigorous studies are now underway in order to determine the origin of this unusual specificity of C-NAD for ADH.

Conclusions

The C-NAD, an isosteric analogue of the natural coenzyme, was found to be an extremely selective inhibitor of alcohol dehydrogenase (nanomolar versus micromolar range) in comparison to other dehydrogenases. If similar selectivity is found for IMPDH then such NAD analogue may be valuable in cancer treatment. With the exception of tiazofurin, other nucleosides related to nicotinamide riboside are not effectively metabolized into their corresponding NAD analogues, as this study showed. Therefore, NAD analogues, *not nucleosides*, that are able to penetrate cells membrane may be of therapeutic interest. They do not require metabolic activation by cellular enzymes. Further studies are in progress.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Column chromatography was performed on silica gel G60 (70–230 mesh, ASTM, Merck). TLC was performed on Analtech Uniplates with short wavelength for visualization. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. ^1H and ^{31}P NMR spectra were recorded on a Bruker AMX-250 and -400 spectrometer with Me_4Si or DDS as the internal standards. Chemical shifts are reported in ppm (δ) and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad singlet), and dd (double doublet). Values given for coupling constants are first order.

Methyl 6-(2,3:4,5-Di-*O*-isopropylidene-D-*altro*-pentitol-1-yl)picolinate (16). To a solution of 6-(2,3:4,5-di-*O*-isopropylidene-D-*altro*-pentitol-1-yl)-2-bromopyridine[36] (*altro*-14, 250 mg, 0.66 mmol) in ethyl ether (15 mL) was added a solution of *n*-BuLi (1.1 mL of a 2.5 M solution in hexane) under argon atmosphere at −78 °C. The mixture was stirred at −78 °C for 25 min, and then a large excess of CO_2 was bubbled through the reaction mixture, which was allowed to reach room temperature. After addition of water (10 mL), the organic layer was separated, and the aqueous layer was acidified with 1 N HCl to pH = 4 and then extracted with $CHCl_3$ (4 × 20 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give a crude picolinic acid derivative (90 mg, 40%), which was dissolved in MeOH (0.5 mL) and treated with ethereal solution of diazomethane (0.8 mmol) at 0 °C. The mixture was allowed to warm to room temperature, and excess CH_2N_2 was decomposed by addition of CH_3COOH. The reaction mixture was concentrated in vacuo, and the residue was chromatographed on a silica gel column using CH_2Cl_2, followed by CH_2Cl_2–MeOH (0.5%) and CH_2Cl_2–MeOH (1%) to give *altro*-16 (87 mg, 90%) as an oil: ^1H NMR ($CDCl_3$) δ 1.29 (s, 3H, iso-Pr), 1.35 (s, 3H, iso-Pr), 1.41 (s, 3H, iso-Pr), 1.47 (s, 3H, iso-Pr), 3.94 (dd, 1H, H5′, $J_{5',5''}$ = 8.6 Hz, $J_{4',5'}$ = 5.8 Hz), 3.99 (s, 3H, COOMe), 4.16–4.23 (m, H3′, H5″), 4.63 (dt, 1H, H4′, $J_{3',5'}$ = 10.2 Hz, $J_{4',5'}$ = $J_{4',5''}$ = 5.8 Hz), 4.75 (dd, 1H, H2′, $J_{2',3'}$ = 6.4 Hz), 5.27 (d, 1H, $J_{1',2'}$ = 1.7 Hz), 7.75 (dd, 1H, H5, $J_{4,5}$ = 7.7 Hz, $J_{3,5}$ = 1.1 Hz), 7.86 (t, 1 H, H4, $J_{3,4}$ = 7.7 Hz), 8.03 (dd, 1 H, H3). Anal. ($C_{18}H_{25}NO_7$) C, H, N.

6-(1-*O*-Mesyl-2,3:4,5-di-*O*-isopropylidene-D-*altro*-pentitol-1-yl)picolinamide (18). Compound *altro*-16 (63 mg, 0.17 mmol) was kept in NH_3/MeOH (10 mL) overnight, and the reaction mixture was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (2 mL), and (dimethylamino)pyridine (2 mg), Et_3N (0.13 mL), and then CH_3SO_2Cl (0.06 mL) were added. The mixture was stirred at room temperature for 2 h, diluted with CH_2Cl_2 (8 mL), and washed with 5% $NaHCO_3$ (3 × 5 mL) and water (2 × 5 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo, and the residue was chromatographed on a silica gel column using $CHCl_3$–EtOH (1%) as the eluent to give *altro*-18 (140 mg, 62%) as a foam: ^1H NMR ($CDCl_3$) δ 0.89 (s, 3H, iso-Pr), 1.17 (s, 3H, iso-Pr), 1.42 (s, 3H, iso-Pr), 1.53 (s, 3H, iso-Pr), 3.08 (s, 3H, OMs), 3.88 (dd, 1H, H5′, $J_{4',5'}$ = 5.0 Hz, $J_{5',5''}$ = 8.8 Hz), 4.03 (dd, 1H, H3′, $J_{3',4'}$ = 9.9 Hz), 4.11 (dd, 1H, H5″, $J_{4',5''}$ = 6.2 Hz), 4.20–4.31 (m, 1H, H4′), 4.98 (dd, 1H, H2′, $J_{2',3'}$ = 7.2 Hz), 5.70 (br s, 1H, NH_2), 5.98 (d, 1H, H1′, $J_{1',2'}$ = 7.2 Hz), 7.70 (br s, 1H, NH_2), 7.73 (dd, 1H, H5, $J_{4,5}$ = 7.7 Hz, $J_{3,5}$ = 1.1 Hz), 7.93 (t, 1H, H4, $J_{3,4}$ = 7.7 Hz), 8.21 (dd, 1H, H3). Anal. ($C_{18}H_{26}N_2O_8S$) C, H, N.

In the similar manner, but without isolation of the derivative *allo*-16, the corresponding *allo*-mesyl derivative 18 was obtained in 18% yield starting from the *allo*-14: ^1H NMR ($CDCl_3$) δ 1.30 (s, 3H, iso-Pr), 1.31 (s, 3H, iso-Pr), 1.37 (s, 3H, iso-Pr), 1.39 (s, 3H, iso-Pr), 2.86 (s, 3H, OMs), 3.94 (dd, 1H, H5′, $J_{4',5'}$ = 5.8 Hz, $J_{5',5''}$ = 8.8 Hz), 4.15–4.22 (m, 2H, H3′,5″), 4.47 (dt, 1H, H4′, $J_{3',4'}$ = 9.4 Hz, $J_{4',5''}$ = 5.8 Hz), 4.79 (dd, 1H, H2′, $J_{2',3'}$ = 5.5 Hz), 5.65 (br s, 1H, NH_2), 5.87 (d, 1H, H1′, $J_{1',2'}$ = 7.1 Hz), 7.69 (dd, 1H, H5, $J_{4,5}$ = 7.7 Hz, $J_{3,5}$ = 1.0 Hz), 7.79 (br s, 1H, NH_2), 7.94 (t, 1H, H4, $J_{3,4}$ = 7.7 Hz), 8.21 (dd, 1H, H3). Anal. ($C_{18}H_{26}N_2O_8S$) C, H, N.

6-(β-D-Ribofuranosyl)picolinamide (8). A solution of *altro*-18 (390 mg, 0.91 mmol) in a mixture of $CF_3COOH/CHCl_3$ (4:1, v/v, 6.3 mL) was stirred at room temperature for 1 h and then diluted with water (10 mL). The aqueous layer was separated, washed with ethyl ether (3 × 4 mL), and concentrated in vacuo. The residue was purified on a column of Dowex 50W-X8 (H^+) using water as the eluent to give 8 (150 mg, 65%): ^1H NMR (Me_2SO-d_6/D_2O) δ 3.52 (dd, 1H, H5′, $J_{4',5'}$ = 4.3 Hz, $J_{5',5''}$ = 11.9 Hz), 3.61 (dd, 1H, H5″, $J_{4',5''}$ = 3.7 Hz), 3.87 (q, 1H, H4′), 3.91 (t, H3′, $J_{3',4'}$ = 5.0 Hz), 4.04 (pseudo t, 1H, H2′, $J_{2',3'}$ = 5.0 Hz), 4.76 (d, 1H, H1′, $J_{1',2'}$ = 5.4 Hz), 7.65 (br s, 1H, NH_2), 7.73 (dd, 1H, H5, $J_{3,5}$ = 1.7 Hz, $J_{4,5}$ = 7.1 Hz), 7.80–7.99 (m, 2 H, H3, H4), 8.19 (br s, 1H, NH_2). Anal. ($C_{12}H_{14}N_2O_5$) C, H, N.

6-(α-D-Ribofuranosyl)picolinamide. The same treatment of the *allo*-18 with a mixture of $CF_3COOH/CHCl_3$ afforded the α-anomer of 8 in 69% yield as white foam: ^1H NMR (Me_2SO-d_6/D_2O) δ 3.48 (dd, 1H, H5′, $J_{4',5'}$ = 4.6 Hz, $J_{5',5''}$ = 12.0 Hz), 3.68 (dd, 1H, H5″, $J_{4',5''}$ = 2.4 Hz), 3.92–3.98 (m, 1H, H4′), 4.14 (dd, 1H, H3′, $J_{3',4'}$ = 8.1 Hz), 4.22 (pseudo t, 1H, H2′, $J_{2',3'}$ = 4.3 Hz), 5.03 (d, 1H, H1′, $J_{1',2'}$ = 3.3 Hz), 7.58 (br s, 1H, NH_2), 7.59 (dd, 1H, H5, $J_{4,5}$ = 7.6 Hz, $J_{3,5}$ = 1.3 Hz), 7.86 (dd, 1H, H3, $J_{3,4}$ = 7.6 Hz), 7.92 (t, 1H, H4) 8.00 (br s, 1H, NH_2). Anal. ($C_{11}H_{14}N_2O_5$) C, H, N.

5-(β-D-Ribofuranosyl)nicotinamide-(5′-5″)-adenosine Pyrophosphate (11). To a suspension of 5-(β-D-ribofuranosyl)-nicotinamide[35] (7, 100 mg, 0.4 mmol) in $(C_2H_5O)_3PO$ (0.4 mL) was added $POCl_3$ (72 mg, 0.48 mmol) at 0 °C, and the mixture was stirred at room temperature for 4 h. The reaction was quenched by addition of water (5 mL), and the mixture was neutralized with concentrated NH_3. The crude product was purified on a column of DEAE Sephadex A-25 (bicarbonate form) with 0.1 M TEAB and then on a Dowex 50W-X8 (H^+) column with water to give the nucleotide (76 mg). This compound was dried by coevaporation with pyridine (3 × 5 mL) and DMF (3 × 5 mL) and then dissolved in DMF (0.7 mL). Carbonyldiimidazole (186 mg, 1.15 mmol) was added, and the progress of reaction was followed by TLC (iPrOH–concentrated NH_4OH–H_2O, 6:3:1, v/v/v). The excess of CDI was hydrolyzed by addition of MeOH (76 μL), and a solution of AMP (126 mg, 0.35 mmol) in DMF (4.4 mL) containing Bu_3N (80 μL, 0.35 mmol) was added. The reaction mixture was stirred for 3 days. Water (10 mL) was added, and the mixture was concentrated in vacuo. The gummy residue was dissolved in water (40 mL) containing NaOAc (60 mg) and extracted with $CHCl_3$ (2 × 40 mL) and Et_2O (2 × 40 mL). The aqueous layer was treated with Et_3N (60 mL, pH = 10) and then lyophilized. The residue was purified on preparative cellulose plate using iPrOH–concentrated NH_3–H_2O (6:3:1) and then by a column of Dowex 50W-X8 (H^+) to give 11 (90 mg, 60%) as a white powder: ^1H NMR (D_2O) δ 4.11–4.41 (m, 8H, H3′, H3″, H4′, H4″, H5′, H5′, H5″, H5″), 4.54 (pseudo t, 1H, H2′), 4.76 (pseudo t, 1H, H2″), 5.06 (d, 1H, H1′, $J_{1',2'}$ = 7.2 Hz), 6.14 (d, 1H, H1″, $J_{1'',2''}$ = 5.2 Hz), 8.43, 8.62 (2 1H singlets, H2, H8), 8.95–9.19 (m, 5H, H2, H4, H6, NH_2); MS (FAB) *m/e* 662 ($M - H)^-$, 664 MH^+.

6-(β-D-Ribofuranosyl)picolinamide-(5′-5″)-adenosine Pyrophosphate (12). Nucleoside 8 (100 mg) was treated with $POCl_3$ to give the 5′-monophosphate, which was activated with carbonyldiimidazole and coupled with AMP as described above. The crude product was purified first on DEAE Sephadex A-25 (bicarbonate form) with a linear gradient of water–0.1 M tetraethylammonium bicarbonate (TEAB) and then by HPLC on a Dynamax-300A C18 column with 0.1 M TEAB followed by

a linear gradient of 0.1 M TEAB–aqueous CH_3CN (70%). Finally, the compound was converted into the sodium salt by passing through a column of Dowex 50W-X8 (Na^+). The overall yield was 50%: ^1H NMR (D_2O) δ 4.10–4.40 (m, 8H, H3′, H3″, H4′, H4″, H5′, H5′, H5″, H5″), 4.45 (pseudo t, 1H, H2′), 4.61 (pseudo t, 1H, H2″), 4.80 (br s, H1′, H_2O), 5.99 (d, 1H, H2″, $J_{1'',2''}$ = 5.3 Hz), 7.59 (d, 1H, H5, $J_{4,5}$ = 6.9 Hz), 7.69 (d, 1H, H3, $J_{3,4}$ = 6.7 Hz), 7.81 (t, 1H, H4), 8.11, 8.34 (2 1H singlets, H2, H8); MS (FAB) *m/e* 662 $(M - H)^-$, 664 MH^+.

Study of IMP Dehydrogenase (IMPDH) Activity. Murine leukemia L1210 cells growing in logarithmic phase in RPMI 1640 medium containing 10% fetal bovine serum in an atmosphere of air and 5% CO_2 at 37 °C were harvested by centrifugation at 400*g*, and the cells were washed once with cold phosphate buffer saline.[39] The cell pellet was sonicated (3 × 5 s) in 4 volumes of cold 40 mM Tris-HCl buffer containing 25% sucrose, 1 mM EDTA, and 1 nM dithiothreitol. The extract was centrifuged at 100000*g* for 30 min at 4 °C and the supernatant dialyzed for 16 h at 4 °C against two changes of the same homogenizing buffer and then used as the source of enzyme. IMPDH activity was assayed by using [2-^{3}H]IMP (Moravek Biochemocals Inc, Brea, CA) by the methodology already described.[40] The activity was expressed as *n* mol of XMP formed/h per mg of protein.

Cytotoxicity of C-NAD to Murine Leukemia L1210 Cells. Murine leukemia L1210 cells were grown in RPMI 1640 medium as detailed above. Logarithmically growing cells were incubated with various concentrations of C-NAD for 24- and 48-h periods, and the cytotoxicity determined by counting the cells in a Coulter counter by the published technique.[39]

Inhibition of Horse Liver Alcohol Dehydrogenase (ADH) and Bovine Glutamate Dehydrogenase (GDH). Inhibition constants (K_i) were determined using NAD as the variable substrate. Rate measurements were made by monitoring the increase in absorbance at 340 nm resulting from the reduction of NAD to NADH, as described previously.[12]

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References

(1) NAD analogues 1.
(2) *The Pyridine Nucleotide Coenzymes*; Anderson, B. M., Everse, J., You, Y.-S., Eds.; Academic Press: New York, 1982.
(3) *Pyridine Nucleotide Coenzymes*; Dolphin, D., Avramovic, O., Poulson, R., Eds.; Wiley: New York, 1987.
(4) Muller-Steffner, H. M.; Malver, O.; Hosie, L.; Oppenheimer, N. J.; Schuber, F. Slow-binding Inhibition of NAD^+ Glycohydrolase by Arabino Analogues of β-NAD^+. *J. Biol. Chem.* **1992**, *267*, 9606–9611.
(5) Sleath, P. R.; Handlon, A. L.; Oppenheimer, N. J. Pyridine Coenzyme Analogues. 3. Synthesis of Three NAD^+ Analogues Containing a 2′-Deoxy-2′-substituted Nicotinamide Arabinofuranosyl Moiety. *J. Org. Chem.* **1991**, *56*, 3608–3613.
(6) Kam, B. L.; Malver, O.; Marschner, T. M.; Oppenheimer, N. J. Pyridine Coenzyme Analogues. Synthesis and Characterization of α- and β-Nicotinamide Arabinoside Adenine Dinucleotides. *Biochemistry* **1987**, *26*, 3453–3461.
(7) Slama, J. T.; Simmons, A. M. Carbanicotinamide Adenine Dinucleotide: Synthesis and Enzymological Properties of a Carbocyclic Analogue of Oxidized Nicotinamide Adenine Dinucleotide. *Biochemistry* **1988**, *27*, 183–193.
(8) Slama, J. T.; Simmons, A. M. Inhibition of NAD Glycohydrolase and ADP-ribosyl Transferases by Carbocyclic Analogues of Oxidized Nicotinamide Adenine Dinucleotide. *Biochemistry* **1989**, *28*, 7688–7694.
(9) Gebeyehu, G.; Marquez, V. E.; Kelley, J. A.; Cooney, D. A.; Jayaram, H. N.; Johns, D. G. Synthesis of Thiazole-4-carboxamide Adenine Dinucleotide. A Powerful Inhibitor of IMP Dehydrogenase. *J. Med. Chem.* **1983**, *26*, 922–925.
(10) Gebeyehu, G.; Marquez, V. E.; Van Cott, A.; Cooney, D. A.; Kelly, J. A.; Jayaram, H. N.; Ahluwalia, G. S.; Dion, R. L.; Wilson, Y. A.; Johns, D. G. Ribavirin, Tiazofurin, and Selenazofurin: Mononucleotides and Nicotinamide Dinucleotide Analogues. Synthesis, Structure, and Interactions with IMP Dehydrogenase. *J. Med. Chem.* **1985**, *28*, 99–105.
(11) Marquez, V. E.; Tseng, C. K. H.; Gebeyehu, G.; Cooney, D. A.; Ahluwalia, G. S.; Kelley, J. A.; Dalal, M.; Fuller, R. W.; Wilson, Y. A.; Johns, D. G. Thiazole-4-carboxamide Adenine Dinucleotide (TAD). Analogues Stable to Phosphodiesterase Hydrolysis. *J. Med. Chem.* **1986**, *29*, 1726–1731.
(12) Goldstein, B. M.; Bell, J. E.; Marquez, V. E. Dehydrogenase Binding by Tiazofurin Anabolites. *J. Med. Chem.* **1990**, *33*, 1123–1127.
(13) Jayaram, H. N.; Dion, R. L.; Glazer, R. J.; Johns, D. G.; Robins, R. K.; Srivastawa, P. C.; Cooney, D. A. Initial Studies on the Mechanism of Action of a New Oncolytic Thiazole Nucleoside, 2-β-D-ribofuranosylthioazole-4-carboxamide. *Biochem. Pharmacol.* **1982**, *31*, 2371–2380.
(14) Yamada, Y.; Natsumeda, Y.; Weber, G. Action of the Active Metabolites of Tiazofurin and Ribavirin on Purified IMP Dehydrogenase. *Biochemistry* **1988**, *27*, 2193–2196 and references therein.
(15) Jackson, R. C.; Weber, G.; Morris, H. P. IMP Dehydrogenase – an Enzyme Linked With Proliferation and Malignancy. *Nature* **1975**, *256*, 331–333.
(16) Weber, G.; Prajda, N.; Jackson, R. C. Key Enzymes of IMP Metabolism: Transformation- and Peroliferation-Linked Alterations in Gene Expression. *Adv. Enzyme Regul.* **1986**, *14*, 3.
(17) Becher, H. J.; Lohr, G. W. Inosine-5′-phosphate Dehydrogenase Activity in Normal and Leukemic Blood Cells. *Klin. Wochenschr.* **1979**, *57*, 1109–1115.
(18) Robins, R. K. Nucleoside and Nucleotide Inhibitors of Inosine Monophosphate (IMP) Dehydrogenase as Potential Antitumor Inhibitors. *Nucleosides Nucleotides* **1982**, *1*, 35–44.
(19) Jackson, R. C.; Morris, H. P.; Weber, G. Partial Purification, Properties and Regulation of Inosine-5′-phosphate Dehydrogenase in Normal and Malignant Rat Tissues. *Biochem. J.* **1977**, *166*, 1–10.
(20) Goldstein, B. M.; Leary, J. F.; Farley, B. A.; Marquez, V. E.; Levy, P. C.; Rowley, P. T. Induction of HL60 Cell Differentiation by Tiazofurin and Its Analogues: Characterization and Efficacy. *Blood* **1991**, *78*, 593–598.
(21) Robins, R. K.; Srivastawa, P. C.; Narayanan, V.; Plowman, J.; Paull, K. 2-β-D-Ribofuranosylthiazole-4-carboxamide, a Novel Potential Antitumor Agent for Lung Tumors and Metastases. *J. Med. Chem.* **1982**, *35*, 107–108.
(22) Carney, D. N.; Ahluwalia, G. S.; Jayaram, H. N.; Cooney, D. A.; Johns, D. G. Relationship Between the Cytotoxicity of Tiazofurin and Its Metabolism by Cultured Human Lung Cancer Cells. *J. Clin. Invest.* **1985**, *75*, 175–182.
(23) Jacobsen, S. J.; Page, T.; Diala, E. S.; Nyhan, W. L.; Robins, R. K.; Mangum, J. H. Synergistic Activity of Purine Metabolism Inhibitors in Cultured Human Tumor Cells. *Cancer Lett.* **1987**, *35*, 97–99.
(24) Fridland, A.; Conelly, M. C.; Robbins, T. J. Tiazofurin Metabolism in Human Lymphoblastoid Cells: Evidence for Phosphorylation by Adenosine Kinase and 5′-Nucleotidase. *Cancer Res.* **1986**, *46*, 532–537.
(25) Ahluwalia, G. S.; Jayaram, H. N.; Plowman, J. P.; Cooney, D. A.; Johns, D. G. Studies on the Mechanism of Action of 2-β-D-Ribofuranosylthiazole-4-carboxamide. *Biochem. Pharmacol.* **1984**, *33*, 1195–1203.
(26) Srivastava, P. C.; Robins, R. K. Synthesis and Antitumor Activity of 2-β-D-Ribofuranosylselenazole-4-carboxamide and Related Derivatives. *J. Med. Chem.* **1983**, *26*, 445–448.
(27) Boritzki, T. J.; Berry, D. A.; Besserer, J. A.; Cook, P. D.; Fry, D. W.; Leopold, W. R.; Jackson, R. C. Biochemical and Antitumor Activity of Tiazofurin and Its Selenium Analogue. *Biochem. Pharmacol.* **1985**, *34*, 1109–1114.
(28) Franchetti, P.; Cristalli, G.; Grifantini, M.; Cappellacci, L.; Sauro, V.; Nocentini, G. Synthesis and Antitumor Activity of 2-β-D-Ribofuranosyloxazole-4-carboxamide. *J. Med. Chem.* **1990**, *33*, 2849–2852.
(29) Goldstein, B. M.; Mao, D. T.; Marquez, V. E. Ara-tiazofurin: Conservation of Structural Features in an Unusual Thiazole Nucleoside. *J. Med. Chem.* **1988**, *31*, 1026–1031.
(30) Mao, D. T.; Marquez, V. E. Synthesis of 2-β-D-Ara- and 2-β-D-Xylofuranosylthiazole-4-carboxamide. *Tetrahedron Lett.* **1984**, *25*, 2111–2114.
(31) Hennen, W. J.; Robins, R. K. Synthesis of 5-(β-D-Ribofuranosyl)-1,2,4-oxadiazole-3-carboxamide. *J. Heterocycl. Chem.* **1985**, *22*, 1747–1748.
(32) Riley, T. A.; Hennen, W. J.; Kent Dalley, N.; Wilson, B. E.; Robins, R. K.; Larson, S. B. Synthesis of 2-(β-D-Ribofuranosyl)pyrimidines, A New Class of C-Nucleosides. *J. Heterocycl. Chem.* **1987**, *24*, 955–964.
(33) Bergstrom, D. E.; Zhang, P. An Efficient Route to C-4-Linked Imidazole Nucleosides: Synthesis of 2-Carbamoyl-4-(2′-Deoxy-β-D-ribofuranosyl)imidazole. *Tetrahedron Lett.* **1991**, *32*, 6485–6487.

(34) Kabat, M. M.; Pankiewicz, K. W.; Watanabe, K. A. Synthesis of 5-β-D-Ribofuranosylnicotinamide and Its N-Methyl Derivative. The Isosteric and Isoelectronic Analogues of Nicotinamide Nucleoside. *J. Med. Chem.* **1987**, *30*, 924–927.

(35) Pankiewicz, K. W.; Sochacka, E.; Kabat, M. M.; Ciszewski, L. A.; Watanabe, K. A. Efficient Synthesis of 5-(β-D-Ribofuranosyl)-nicotinamide and Its α-Isomer. *J. Org. Chem.* **1988**, *53*, 3473–3479.

(36) Kabat, M. M.; Pankiewicz, K. W.; Sochacka, E.; Watanabe, K. A. Nucleosides CXLVIII. Synthesis of 6-(β-D-Ribofuranosyl)picolinamide. A Novel C-Nucleoside from D-Ribonolactone. *Chem. Pharm. Bull.* **1988**, *36*, 634–640.

(37) Joos, P. E.; Esmans, E. L.; Dommisse, R. A.; Van Dongen, W.; Lepoivre, J. A.; Alderweireldt, F. C.; Balzarini, J.; De Clerq, E. Synthesis and Biological Evaluation of Carbamoyl-2-β-D-ribofuranosylpyridine. *Nucleosides Nucleotides* **1991**, *10*, 883–894.

(38) Krohn, K.; Heins, H.; Wielckens, K. Synthesis and Cytotoxic Activity of C-glycosidic Nicotinamide Riboside Analogues. *J. Med. Chem.* **1992**, *35*, 511–517.

(39) Jayaram, H. N. Biochemical Mechanism of Resistance to Tiazofurin. *Adv. Enzyme Regul.* **1984**, *24*, 67–89.

(40) Cooney, D. A.; Wilson, Y.; McGee, E. A Straightforward Radiometric Technique for Measuring IMP Dehydrogenase. *Anal. Biochem.* **1983**, *130*, 339–345.